USER GUIDE

Encore® SP Rapid Library Systems

PART NOS. 8041 AND 8042

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I. Introduction

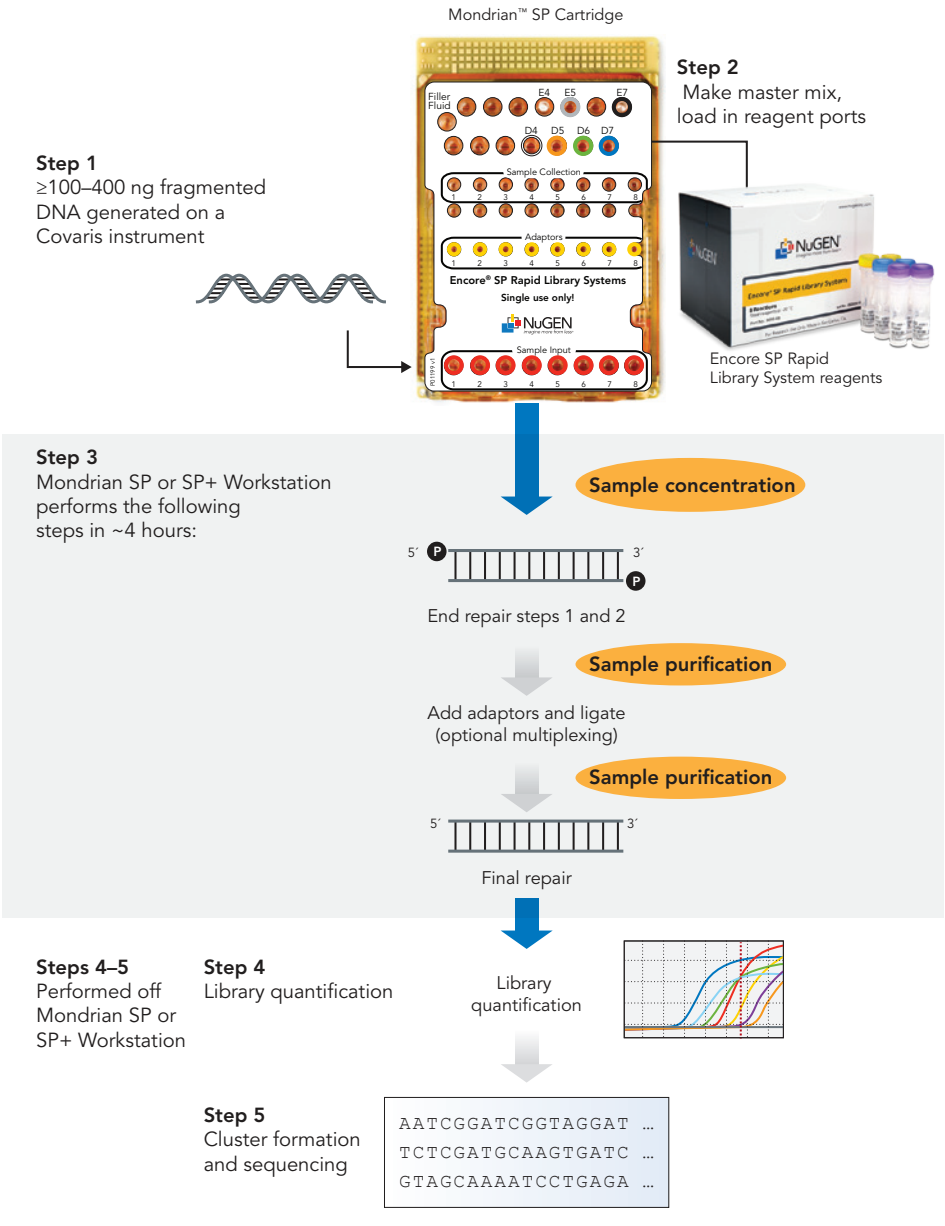
A. Background

The Encore® SP Rapid Library Systems, composed of Encore SP Rapid DR Multiplex Systems 1–8 and 9–16 (Part Nos. 8041 and 8042), are complete reagent, cartridge and protocol packages for the simple automation of DNA library preparation protocols for next generation sequencing using the Mondrian™ SP or SP+ Workstation. These systems enable library preparation from 100–400 ng of sheared, double-stranded DNA (dsDNA) and do not require any post-Mondrian PCR enrichment or purification prior to library quantitation and sequencing. The resulting libraries are suitable for a range of sequencing applications including RNA-Seq, Digital Gene Expression (DGE), genomic DNA sequencing and amplicon sequencing. As shown in Figure 1, the streamlined workflow consists of five steps:

1. Fragmentation of either genomic DNA or double-stranded cDNA to produce the assay template
2. Addition of the template and reagents to the Mondrian SP Universal Cartridge (see Figure 1A)
3. Hands-free automation of the following assay steps on the Mondrian SP or SP+ Workstation (see Figure 1B):
   - Sample concentration
   - End repair
   - Sample purification
   - Adaptor ligation (with optional sample multiplexing)
   - Sample purification
   - Final repair
4. Library quantification with KAPA Library Quantification Kit
5. Cluster formation and sequencing (see Figure 1C)

I. Introduction

Figure 1. The five steps of the Encore SP Rapid Library System workflow. A) Steps 1–2: Covaris fragmentation of the dsDNA template and loading the Mondrian SP Cartridge with template and reagents. B) Step 3: assay steps automated on the Mondrian SP Cartridge. C) Steps 4–5: library quantification, cluster formation and sequencing.



I. Introduction

The entire workflow requires no manual bead purification or gel purification steps. Starting with 100–400 ng of fragmented double-stranded DNA (dsDNA), the protocol can be completed in approximately 4 hours and yields libraries ready for quantification prior to cluster formation and either single read or paired-end sequencing.

Importantly for DNA sequencing applications, samples can be input directly to the library construction workflow without the need for pre-amplification. The absence of PCR steps in the library construction workflow makes this library construction method particularly well suited to either high- or low-GC genomes, which are often susceptible to PCR artifacts.

The Encore SP Rapid DR Multiplex Systems 1–8 and 9–16 each provide eight unique dedicated read barcoded adaptors to prepare libraries for multiplex sequencing using a dedicated read design strategy with a second sequencing primer. Together, these two multiplex kits enable up to 16-plex sequencing.

Important: Please familiarize yourself with the instructions in the Mondrian SP Universal Cartridge User Guide (Part No. M01265) and the Mondrian SP+ Workstation User Manual (Part No. M01322) prior to beginning any work with the Encore SP Rapid Library Systems.

B. Performance Specifications

The Encore SP Rapid Library Systems are designed to produce DNA libraries suitable for either single read or paired-end sequencing on the Illumina Genome Analyzer IIx/IIe (GAII), MiSeq, HiScan SQ or HiSeq NGS platforms without gel-based size selection, using 100 to 400 ng input of double-stranded DNA. The absence of PCR amplification in the workflow eliminates the risk of unequal amplification or PCR bias introduced as a result of differences in template composition. The Encore SP Rapid Library Systems generate quantified libraries ready for cluster generation on an Illumina System in about 7 hours.

C. Library Quantification

Libraries created using Encore Rapid Library Systems must be quantified using qPCR. These libraries cannot be accurately quantified using other means. We recommend using KAPA Library Quantification kits from KAPA Biosystems for quantification. See Section V. Quantitative and Qualitative Assessment of the Purified Libraries for details.

D. Quality Control

Every lot of the Encore SP Rapid Library Systems undergoes functional testing to confirm that the products meet the specifications for library generation performance.

I. Introduction

We recommend the use of control samples when beginning experiments and/or using a new source of samples. For RNA-based experiments (such as RNA-Seq) we recommend the use of the MicroArray Quality Control (MAQC) reference samples A and B. For DNA-based experiments (such as WGS) we recommend the use of a control DNA sample from the HapMap project.

E. Storage and Stability

This product contains components with multiple storage temperatures.

The Encore SP Rapid Library Systems reagents are shipped in two boxes. Box 1 is shipped on dry ice and should be stored at –20°C on an internal shelf of a freezer without a defrost cycle upon receipt. Box 2 is shipped and should be stored at room temperature. The Agencourt® RNAClean® XP Beads (clear cap) are shipped separately and should be removed from the top of the Box 2 shipping carton upon delivery and stored at 4°C.

The kit has been tested to perform to specifications after as many as four freeze/thaw cycles. Kits handled and stored according to the above guidelines will perform to specifications for at least six months.

F. Material Safety Data Sheet (MSDS)

An MSDS for this product is available on the NuGEN website at http://www.nugen.com/nugen/index.cfm/support/user-guides/.

II. Components

A. Reagents Provided

Table 1. Encore SP Rapid DR Multiplex System 1–8 Components and Reagents (Part No. 8041-32)

8041 PART NUMBER	8041 DESCRIPTION	8041 BOX	8041 VIAL CAP	8041 VIAL NUMBER
S01705	End Repair Buffer Mix	1 of 2	Blue	ER1 VER 6
S01706	End Repair Enzyme Mix	1 of 2	Blue	ER2 VER 4
S01626	End Repair Enhancer	1 of 2	Blue	ER3
S01625	End Repair Enhancer Buffer Mix	1 of 2	Blue	ER4
S01662	Ligation Buffer Mix	1 of 2	Yellow	L1 VER 5
S01669 S01670 S01671 S01672 S01673 S01674 S01675 S01676	DR Multiplex Ligation Adaptors 1–8	1 of 2	Yellow	L2V9DR-BC1 L2V9DR-BC2 L2V9DR-BC3 L2V9DR-BC4 L2V9DR-BC5 L2V9DR-BC6 L2V9DR-BC7 L2V9DR-BC8
S01467	Ligation Enzyme Mix	1 of 2	Yellow	L3 VER 4
S01703	Final Repair Buffer Mix	1 of 2	Purple	FR1 VER 2
S01704	Final Repair Enzyme Mix	1 of 2	Purple	FR2 VER 2
S01707	Library Dilution Buffer Mix	1 of 2	N/A	LD1
S01001	Nuclease-free Water	1 of 2	Green	D1
P01190	Mondrian SP Cartridges (X4)	2 of 2	N/A	N/A
S01561	Cartridge Filler Fluid (X4)	2 of 2	N/A	N/A
S01556	Sample Concentration Solution	2 of 2	N/A	N/A
S01698	Agencourt RNAClean XP Purification Beads	Shipped separately	N/A	N/A
S01588	Bead Binding Solution	2 of 2	N/A	N/A

Encore SP Rapid DR Multiplex System 1–8 Components and Reagents (Part No. 8041-32) *continued*				
8041 PART NUMBER	**8041 DESCRIPTION**	**8041 BOX**	**8041 VIAL CAP**	**8041 VIAL NUMBER**
S01589	Bead Wash Solution	2 of 2	N/A	N/A
S01590	Elution Buffer	2 of 2	N/A	N/A
P01199	Encore SP Rapid Cartridge Loading Guide (X4)	2 of 2	N/A	N/A

Table 2. Encore SP Rapid DR Multiplex System 9–16 Components and Reagents (Part Number 8042-32)

8042 PART NUMBER	8042 DESCRIPTION	8042 BOX	8042 VIAL CAP	8042 VIAL NUMBER
S01705	End Repair Buffer Mix	1 of 2	Blue	ER1 VER 6
S01706	End Repair Enzyme Mix	1 of 2	Blue	ER2 VER 4
S01626	End Repair Enhancer	1 of 2	Blue	ER3
S01625	End Repair Enhancer Buffer Mix	1 of 2	Blue	ER4
S01662	Ligation Buffer Mix	1 of 2	Yellow	L1 VER 5
S01677 S01678 S01679 S01680 S01681 S01682 S01683 S01684	DR Multiplex Ligation Adaptors 9–16	1 of 2	Yellow	L2V9DR-BC9 L2V9DR-BC10 L2V9DR-BC11 L2V9DR-BC12 L2V9DR-BC13 L2V9DR-BC14 L2V9DR-BC15 L2V9DR-BC16
S01467	Ligation Enzyme Mix	1 of 2	Yellow	L3 VER 4
S01703	Final Repair Buffer Mix	1 of 2	Purple	FR1 VER 2
S01704	Final Repair Enzyme Mix	1 of 2	Purple	FR2 VER 2
S01707	Library Dilution Buffer Mix	1 of 2	N/A	LD1

Encore SP Rapid DR Multiplex System 9–16 Components and Reagents (Part No. 8042-32) *continued*				
8042 PART NUMBER	**8042 DESCRIPTION**	**8042 BOX**	**8042 VIAL CAP**	**8042 VIAL NUMBER**
S01001	Nuclease-free Water	1 of 2	Green	D1
P01190	Mondrian SP Cartridges (X4)	2 of 2	N/A	N/A
S01561	Cartridge Filler Fluid (X4)	2 of 2	N/A	N/A
S01556	Sample Concentration Solution	2 of 2	N/A	N/A
S01698	Agencourt RNAClean XP Purification Beads	Shipped separately	N/A	N/A
S01588	Bead Binding Solution	2 of 2	N/A	N/A
S01589	Bead Wash Solution	2 of 2	N/A	N/A
S01590	Elution Buffer	2 of 2	N/A	N/A
P01199	Encore SP Rapid Cartridge Loading Guide (X4)	2 of 2	N/A	N/A

B. Additional Equipment, Reagents and Labware

Required Materials

- **Equipment**
  - Mondrian SP Workstation (NuGEN Part No. 8000) or Mondrian SP+ Workstation (NuGEN Part No. 8100)
  - Nanodrop® UV-Vis Spectrophotometer or appropriate spectrophotometer and cuvettes for quantification of fragmented DNA
  - Covaris™ S-series Sonication System to fragment input DNA
  - Agilent 2100 Bioanalyzer (optional) for analysis of DNA fragmentation
  - Materials and equipment for electrophoretic analysis of nucleic acids including 2% agarose gels, DNA ladders (1 kB and 50 bp recommended)
  - Real-time PCR system capable of SYBR Green detection
  - Microcentrifuge for individual 1.5 mL and 0.5 mL tubes
  - 0.5–10 µL pipette, 2–20 µL pipette, 20–200 µL pipette
  - Vortexer

II. Components

- **Reagents**
  - 1X TE buffer (low EDTA), pH 8.0 (Affymetrix, Cat. #75793) for input DNA samples
  - KAPA Library Quantification Kit specified for Illumina and the real-time PCR system to be used
  - Quant-iT™ PicoGreen® dsDNA Assay Kit for quantifying fragmented DNA
- **Supplies and Labware**
  - Nuclease-free pipette tips
  - 1.5 mL and 0.5 mL RNase-free microcentrifuge tubes
  - 0.2 mL individual thin-wall PCR tubes or 8 X 0.2 mL strip PCR tubes or 0.2 mL thin-wall PCR plates
  - Disposable gloves
  - Kimwipes
  - Ice bucket
  - Cleaning solutions such as DNA·OFF™ (MP Biomedicals, Cat. #QD0500)

To Order:

- Affymetrix, www.affymetrix.com
- Agilent, www.agilent.com
- Covaris, www.covarisinc.com
- Invitrogen, www.invitrogen.com
- KAPA Biosystems, www.kapabiosystems.com
- MP Biomedicals, www.mpbio.com
- Nanodrop, www.nanodrop.com

III. Planning the Experiment

A. Input DNA Requirements

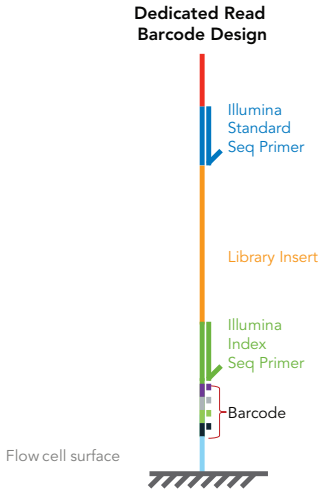
The Encore SP Rapid Library Systems are designed to work with 100 to 400 ng of fragmented genomic DNA or ds-cDNA. DNA samples must be free of contaminating proteins, RNA, organic solvents (including phenol and ethanol) and salts. We recommend using a commercially available system for DNA/cDNA isolation. To obtain optimal library yields, we recommend that the Encore SP Rapid Library System input be determined based on both the fragment size of the sample and the method of quantification. For 200-bp fragments the recommended input is 200 ng (if measured using either NanoDrop or UV absorbance) or 100 ng (if measured using PicoGreen quantification). For 500-bp fragments the recommended input is 350 ng (if measured using either NanoDrop or UV absorbance) or 250 ng (if measured using PicoGreen quantification).

Note: We recommend PicoGreen quantification of the input DNA as it provides greater library yield consistency. If using NanoDrop or UV absorbance, the A260:A280 ratio for DNA samples should be in excess of 1.8. Using DNA samples with lower ratios may result in low library yield.

B. Using Encore SP Rapid Library Systems on Illumina NGS Systems

The Encore SP Rapid Library Systems use a 'Dedicated Read' (DR) design with a second sequencing primer for multiplex sequencing. Figure 2 depicts the DR multiplex barcode strategy.

Figure 2. Dedicated read multiplexing strategy used by the Encore SP Rapid Library Systems.



The Encore SP Rapid DR Multiplex Systems 1–8 and 9–16 use the same approach to multiplexing used in the standard Illumina method. These libraries should be sequenced using the Illumina protocol for multiplex sequencing. The DR barcode sequences are found in Appendix B and must be entered into the Illumina software prior to the analysis.

C. Library Quantification

Libraries created using Encore Rapid Library Systems must be quantified using qPCR. These libraries cannot be accurately quantified using other means. We recommend using KAPA Library Quantification kits from KAPA Biosystems for quantification. See Section V. Quantitative and Qualitative Assessment of the Purified Libraries for details.

D. Final Library Storage

Libraries may be stored at –20°C.

IV. Protocol

A. Overview

The library preparation process used in the Encore SP Rapid Library Systems is performed on the Mondrian SP or SP+ Workstation. The process includes sample concentration, DNA end repair, purification, adaptor ligation and final end repair. It takes approximately 4 hours to complete. The end product is a purified library that is ready for quantification prior to cluster generation on Illumina systems. Library quantification via qPCR takes approximately 3 hours (including qPCR, KAPA product gel and quantification calculations), so the total time to prepare purified and quantified libraries ready for sequencing is approximately 7 hours.

B. Protocol Notes

- The system is designed and intended for processing eight samples at a time. Do not attempt to prepare smaller volume master mixes or process fewer than eight samples using the Encore SP Rapid Library Systems.
- We recommend the routine use of a positive control DNA. Especially the first time you set up a reaction, the use of a positive control DNA will allow you to establish a performance baseline.
- Use the water provided with the kit (green cap, vial D1) or an alternate source of nuclease-free water. We do not recommend the use of DEPC-treated water with this protocol.
- Thaw components used in each step and immediately place them on ice.
- Always keep thawed reagents and reaction tubes on ice unless otherwise instructed.
- After thawing and mixing buffer mixes, if any precipitate is observed, re-dissolve it completely prior to use. You may gently warm the buffer mix for 2 minutes at room temperature followed by brief vortexing. Do not warm any enzyme or primer mixes.
- When placing small amounts of reagents into the reaction mix, pipet up and down several times to ensure complete transfer.
- When instructed to pipet mix, gently aspirate and dispense a volume that is at least half of the total volume of the reaction mix.
- Always allow the thermal cycler to reach the initial incubation temperature prior to placing the tubes or plates in the block.
- Components and reagents from other NuGEN® kits should not be used with the Encore SP Rapid Library Systems.

C. DNA Fragmentation

We recommend using a Covaris S-series instrument to fragment DNA or ds-cDNA to the desired size prior to starting the Encore SP Rapid Library Systems protocol.

1. Dilute appropriate amount of intact DNA into 120 µL of 1X TE buffer (low EDTA), pH 8.0.
2. Transfer 120 µL to Covaris snap cap microtube.

3. Fragment to desired insert size following Covaris recommended settings.
4. Quantify fragmented samples by either UV absorbance or Quant-iT PicoGreen dsDNA assay (preferred).

D. Cartridge Quality Control Check

The Mondrian SP Cartridge QC protocol is a QC check that we recommend be performed prior to adding samples and reagents to the Mondrian SP Cartridge. This QC check confirms the functionality of the Mondrian SP Cartridges prior to use.

The Mondrian SP Cartridge QC protocol requires inserting the Filler Fluid-containing cartridge into the deck of the Mondrian SP or SP+ Workstation. Some customers may find it easier to place the fluid-containing cartridge into the deck of the workstation and then pipet the Elution Buffer into the cartridge. Alternatively, it may be easier to pipet the Elution Buffer into the fluid-containing cartridge (while the cartridge rests on the bench top) and then insert the reagent and fluid-filled cartridge into the deck of the Workstation (moving carefully to avoid spilling the fluid or disturbing the reagent).

1. Locate the Cartridge Loading Guide that is provided with each Mondrian SP Cartridge and place the loading guide on the cartridge (Note: it is only possible to place the loading guide on the cartridge in a single orientation).
2. **Optional:** Carefully move the Filler Fluid-containing cartridge (and its Cartridge Loading Guide) to the deck of the Mondrian SP or SP+ Workstation and insert the cartridge into the deck.
3. Remove the Elution Buffer reagent tube (shipped and stored at room temperature) from the Encore SP Rapid Library System kit:
4. Using a 50- or 100-µL single-channel pipette set to 50 µL, load 50 µL of Elution Buffer into port E5 of the Mondrian SP Cartridge. When adding sample or reagent, lower the pipette tip to the bottom of the port. Do not press the tip into the bottom of the cartridge (if the tip contacts the bottom of the cartridge withdraw the pipette tip slightly upwards). Slowly depress the plunger (to the first stopping point) to dispense the reagent completely from the pipette tip (do not depress the plunger completely as this will introduce bubbles into the cartridge). Once all of the reagent is dispensed from the pipette tip, pull the pipette tip back out of the port and dispose of the tip.

   Note: Do NOT add any samples or other reagents to the cartridge at this time. Ensure that only Elution Buffer has been loaded.
5. If the cartridge is not already inserted into the Mondrian SP or SP+ Workstation deck, carefully transport the cartridge to the Mondrian SP or SP+ Workstation and insert the cartridge into the deck.
6. If not already ON, locate the workstation ON/OFF switch at the back of the workstation and turn ON.

7. Press the 'On' button (Figure 3) on the front of the workstation to turn the instrument on.

Figure 3. The Mondrian SP or SP+ Workstation 'On' button, located on the front of the workstation

8. Pull the cartridge lever of the Mondrian SP or SP+ Workstation forward to the locked position and close the lid of the workstation.
9. Select 'Run' on the touch screen menu, choose the Mondrian SP Cartridge QC protocol from the list of protocols and then select 'Next' to proceed to the Protocol Information screen.
10. Select 'Next' to proceed to the Run Information screen.
11. **Optional:** Enter run details as required in the Run Information screen.
12. Select Next to proceed to the Run Confirmation screen and select 'Start Run'.

The Mondrian SP Cartridge QC protocol will take about nine minutes to complete. During this test, Elution Buffer droplets will be dispensed from the E5 port and transported around the cartridge prior to being discarded. The purpose of this test is to confirm the successful transport of droplets across all lanes of the cartridge.

At the end of the protocol, the instrument will display the Run Complete screen and one of the following messages:

MESSAGE	MEANING	NEXT STEP
Mondrian™ SP cartridge passed. Continue to intended protocol.	No errors detected. Droplet transport was normal.	Press 'OK' on the Run Complete screen to return to the main menu. Proceed to section C: Loading Samples and Reagents (below) in the Mondrian SP Universal Cartridge user guide or follow the instructions (for 3rd party reagents) in the appropriate applications note. The user may remove the cartridge from the Workstation to load samples and remaining reagents on the bench top, taking care that the cartridge remains level at all times. **Note:** It is not necessary to add additional Elution Buffer or Filler Fluid to the cartridge prior to loading reagents and samples.
Mondrian™ SP cartridge failed. Remove cartridge from instrument deck and set aside prior to contacting NuGEN Technical Support.	A problem was detected with droplet transport within the cartridge.	Press 'OK' on the Run Complete screen to return to the main menu. Remove the cartridge from the deck and set it aside (do not discard) and contact NuGEN Technical Support for further instructions. Users who proceed with SP protocols and/or who load additional SP reagents and samples onto failed cartridges are doing so at their own risk and will not be compensated for loss of reagents, samples or cartridges by NuGEN Technologies, Inc.
Mondrian™ SP cartridge status is undetermined. Please consult Mondrian™ SP Universal Cartridge User Guide or appropriate NuGEN® SP Library Systems User Guide for further instructions.	The results are inconclusive and must be repeated one more time prior to making a determination on the quality of the cartridge.	Repeat the Mondrian SP Cartridge QC protocol one more time. • Do not add any additional Filler Fluid or Elution Buffer to the cartridge. • Press OK on the Run Complete screen to return to the main menu. • Proceed to re-run the Mondrian SP Cartridge QC protocol starting from Step 10 in the protocol above. If the message after the second Mondrian SP Cartridge QC protocol is: • 'Mondrian SP cartridge passed,' then proceed as outlined in Next Step for passing cartridges (above). • 'Mondrian SP Cartridge failed' or 'Mondrian SP cartridge status is undetermined,' do not use the cartridge and contact NuGEN Technical Support.

E. Protocol for the Encore SP Rapid Library Systems on Mondrian SP Cartridges

1. Sample Solution Preparation

Prepare sample solution for loading onto the cartridge (this is done on a per sample basis and not as a master mix) according to the volumes shown in Table 3. You must prepare and process no fewer than eight samples on each cartridge.

Table 3. Sample Solution Preparation

COMPONENT	VOLUME
100 to 400 ng sheared ds DNA in water or low EDTA TE	21.4 µL
Agencourt RNAClean XP beads	3.6 µL
Sample Concentration Solution	25 µL
Total volume	**50 µL**

Ensure Agencourt RNAClean XP beads are at room temperature and completely resuspended prior to use. Mix each sample solution well and incubate at room temperature (approximately 23°C) for 10 minutes. The above recipe is meant for one sample. Prepare sample solution for each sample to be processed.

2. Encore SP Rapid Library Systems Reagent Master Mix Preparation

Prepare End Repair Master Mix

1. Thaw End Repair Buffer Mix (ER1 VER 6) at room temperature, vortex to mix well and spin down briefly. Keep End Repair Enzyme Mix (ER2 VER 4) on ice.
2. Prepare master mix in a 0.5 mL microcentrifuge tube or 0.2 mL PCR tube according to the volumes shown in Table 4. Label the tube D6.

IV. Protocol

Table 4. End Repair Master Mix

COMPONENT	VIAL CAP	VOLUME
End Repair Buffer Mix (ER1 VER 6)	Blue	7.0 µL
End Repair Enzyme Mix (ER2 VER 4)	Blue	3.0 µL
Total volume		**10.0 µL**

Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.

Prepare End Repair Enhancer Master Mix

1. Thaw End Repair Enhancer Buffer Mix (ER4) at room temperature, vortex to mix well and spin down briefly. Keep End Repair Enhancer (ER3) on ice.
2. Prepare master mix in a 0.5 mL microcentrifuge tube or 0.2 mL PCR tube according to the volumes shown in Table 5. Label the tube D7.

Table 5. End Repair Enhancer Master Mix

COMPONENT	VIAL CAP	VOLUME
End Repair Enhancer Buffer Mix (ER4)	Blue	7.0 µL
End Repair Enhancer (ER3)	Blue	3.0 µL
Total volume		**10.0 µL**

Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.

Prepare Ligation Master Mix

1. Thaw Ligation Buffer Mix (L1 VER 5) at room temperature, vortex to mix well and spin down briefly. Keep Ligation Enzyme Mix (L3 VER 4) on ice.
2. Prepare master mix in a 0.5 mL microcentrifuge tube or 0.2 mL PCR tube according to the volumes shown in Table 6. Label the tube D5.

Table 6. Ligation Master Mix

COMPONENT	VIAL CAP	VOLUME
Ligation Buffer Mix (L1 VER 5)	Yellow	7.0 µL
Ligation Enzyme Mix (L3 VER 4)	Yellow	3.0 µL
Total volume		**10.0 µL**

Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.

Prepare Final Repair Master Mix

1. Thaw Final Repair Buffer Mix (FR1 VER 2) at room temperature, vortex to mix well and spin down briefly. Keep Final Repair Enzyme Mix (FR2 VER 2) on ice.
2. Prepare master mix in a 0.5 mL microcentrifuge tube or 0.2 mL PCR tube according to the volumes shown in Table 7. Label the tube D4.

Table 7. Final Repair Master Mix

COMPONENT	VIAL CAP	VOLUME
Final Repair Buffer Mix (FR1 VER 2)	Purple	8.0 µL
Final Repair Enzyme Mix (FR2 VER 2)	Purple	2.0 µL
Total volume		**10.0 µL**

! *Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.*

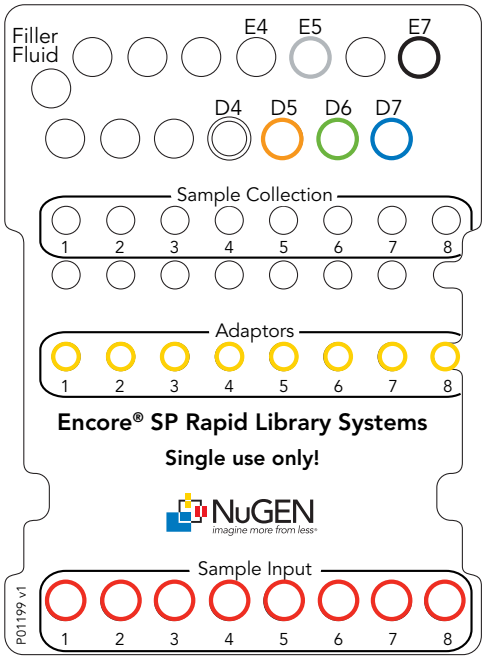
Prepare Adaptors

1. Thaw ligation adaptors at room temperature, vortex well to mix and spin down briefly. Use the desired barcode adaptors L2V9DR-BC1–16 (yellow vial cap).
2. The adaptors will be loaded onto the cartridge as indicated below (Step 8 of the Mondrian SP Cartridge Loading Instructions).

3. Mondrian SP Cartridge Loading Instructions

Important: For detailed instructions on how to prepare the Mondrian SP Cartridge for reagent and sample loading, refer to Mondrian SP Universal Cartridge User Guide (M01265).

Figure 4. Mondrian SP Cartridge loading guide for the Encore SP Rapid Library System protocol.



Loading Reagents and Samples in the Mondrian SP Cartridge

Using Figure 4 as a guide, follow the instructions below to load reagents into their appropriate cartridge ports.

- Use a 10 µL pipette to add all reagent master mixes and the adaptors.

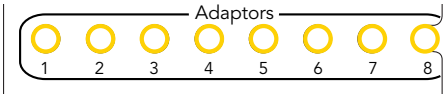
Important: It is critical that a 10 or 20 µL pipette be used for adding any volumes of ≤10 µL. Do not use 2 µL pipettes to add reagents to the cartridge as they will not generate sufficient force to expel the reagents from the pipette tip into the Filler Fluid-containing cartridge.

- Use a 100 or 200 µL pipette for adding the samples, Bead Binding Solution, Elution Buffer and Bead Wash Solution.
- Load the ports in a steady manner to avoid overflow of the Filler Fluid.
- When adding sample or reagent, lower the pipette tip to the bottom of the port. Do not press the tip into the bottom of the cartridge. If the tip contacts

the bottom of the cartridge, withdraw the pipette tip slightly upwards. Slowly depress the plunger to the first stopping point to dispense the reagent completely from the pipette tip. As you raise the pipette tip from the port, you should complete a gentle blow-out by fully depressing the pipette plunger just prior to exiting the Filler Fluid to ensure all the reagent is dispensed.

Using a desktop lamp to illuminate your work area may facilitate sample and reagent loading. We recommend loading the reagents and samples with the cartridge on the deck of the Mondrian SP or SP+ Workstation.

1. Load 1.5 µL of each adaptor mix into ports A1–A8 using a 10 µL pipette. Use L2 VER 10 for all samples. Adaptor ports A1 through A8 are highlighted with yellow rims.



Note: If the adaptor droplet appears to be floating above the surface of the bottom plate of the cartridge, use a clean pipette tip to gently push the droplet down to the surface.

2. Load 8 µL Final Repair Master Mix into D4. Port D4 is highlighted with a white rim circled with a dark line.

3. Load 8 µL Ligation Master Mix into D5. Port D5 is highlighted with an orange rim.

4. Load 8 µL End Repair Master Mix into D6. Port D6 is highlighted with a green rim.

5. Load 8 µL End Repair Enhancer Master Mix into D7. Port D7 is highlighted with a blue rim.

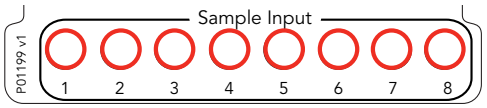
6. Load 50 µL Bead Binding Solution in E4. Port E4 is not highlighted in any way.

7. Load 50 µL Elution Buffer into E5. Port E5 is highlighted with a grey rim.

Note: This step is required only if Elution Buffer was not added during the Cartridge QC Protocol. Do not add additional Elution Buffer if this reagent was already added to the cartridge during the Cartridge QC Protocol.

8. Load 50 µL Bead Wash Solution into E7. Port E7 is highlighted with a black rim.

9. Load 50 µL of sample mix into S1–S8 (ensure that the 10-minute incubation step described above is performed prior to loading onto the cartridge.) Sample input ports are highlighted with red rims.



10. If the cartridge is not already inserted into the Mondrian SP+ Workstation deck, insert the cartridge into the deck and pull the locking lever forward to engage the cartridge with the control electronics and close the lid.

Mondrian SP Workstation Initialization Instructions

1. If not already ON, locate the workstation ON/OFF switch at the back of the workstation and turn ON.
2. Press the 'On' button on the front of the workstation.
3. Select 'Run' on the touch screen menu, select 'Encore SP Rapid' from the list of available protocols and follow the instructions on the screen to begin the run.

Library Collection from the Mondrian SP Cartridge

Note: A short instructional video on 'Mondrian Sample Collection' is available on the Mondrian Support page at www.nugen.com/mondriansupport.

1. Place 8 low-binding 0.2 mL or 0.5 mL microcentrifuge tubes in a rack. Add 11 µL of Library Dilution Buffer (LD1) to each tube.
2. Use a 100 or 200 µL pipette set to 20 µL. Depress the plunger on the pipette and insert the tip all the way to the bottom of the sample collection port, perpendicular to the cartridge to make a seal between the cartridge and the pipet tip.
3. Maintaining the seal formed between the pipette tip and the bottom of the cartridge, release the plunger. Immediately lift the pipette slightly off the bottom of the cartridge to release the seal, rapidly drawing Filler Fluid and the sample droplet into the pipette tip.
4. Examine the pipette tip to ensure that the appropriately sized droplet is suspended in the Filler Fluid.

5. Dispense the collected fluid including the small sample droplet directly into the 11 µL of Library Dilution Buffer in the collection tube. Pipette up and down a few times in the aqueous phase to ensure that all the sample transfers into the buffer.
6. Discard the tip and repeat steps 2–5 to ensure complete collection of the entire sample. This is important because occasionally a portion of the sample droplet is left behind in the sample collection port.
7. Continue to the next sample collection port and repeat this process until all eight libraries have been collected and placed in separate tubes.
8. Remove the cartridge from the workstation and dispose of as appropriate in laboratory waste.
9. Cap the library droplet-containing PCR tubes and vortex briefly to mix. Spin down briefly to bring the aqueous phase to the bottom of the tube. The Filler Fluid oil should remain as a separate layer on top of the aqueous phase.
10. Use a pipette to remove most of the Filler Fluid floating on top of the aqueous phase. This will aid the aqueous phase collection.
11. Use a fresh tip to transfer the aqueous phase (11 µL) from beneath the remaining Filler Fluid and into a fresh tube.
12. The aqueous phase contains the purified library. Store the library at –20°C or proceed immediately to library quantitation.

V. Quantitative and Qualitative Assessment of the Purified Libraries

A. Overview

This section details how to prepare Encore SP Rapid libraries for quantification and qualification prior to cluster generation and sequencing.

Important: Libraries created using Encore Rapid Library Systems must be quantified using qPCR. These libraries cannot be accurately quantified using other means. We recommend using KAPA Library Quantification kits from KAPA Biosystems for quantification.

B. Quantification Using KAPA Biosystems Products

Use the KAPA Library Quantification kit specific for the Illumina NGS platform and your available qPCR instrument. Follow the instructions described by KAPA BioSystems in the Technical Data Sheet for the KAPA Library Quantification kit you are using.

We recommend dilutions be performed with a volume of purified library not to exceed 1.0 µL. Problems may arise if too much sample is used when yields are low.

Note: For the library qPCR reactions, we recommend loading at least duplicates of a 1:1,000 and a 1:10,000 dilution on the same plate as the KAPA Biosystems concentration standards (run in triplicate). See Appendix A for recommendations on the final pMolarity of the hybridization solution.

C. Gel Analysis of KAPA qPCR Product

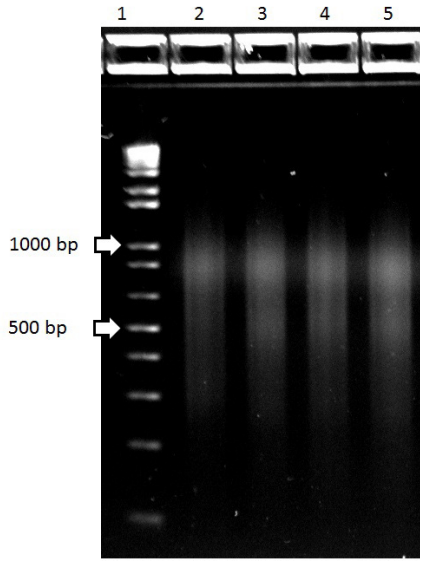
Gel analysis of the library KAPA qPCR product is important for qualitative and quantitative analysis of the final libraries. Use the gel to determine the average size of the library as this value is required for calculation of the concentration of the libraries. Also, use the gel image to determine if adaptor dimers are present. Adaptor dimers may reduce the number of useful sequence reads. On the same 2% agarose gel, run the following lanes:

1. 5 µL of KAPA product from one well of the 1:1,000 dilution and one well of the 1:10,000 dilution for the sample library
2. 5 µL of one KAPA concentration standard
3. 10 µL (10 ng/ µL) of 1 Kb Plus and/or 50 bp ladder

See Figure 5 for an example of KAPA qPCR products (generated with the Encore SP Rapid Library System) run on a 2% agarose gel.

V. Quantitative and Qualitative Assessment of the Purified Libraries

Figure 5. 2% agarose gel analysis of Encore SP Rapid Library System libraries after KAPA qPCR analysis. Lane 1 is the 1 Kb Plus ladder. For size reference, the position of migration of the 1 Kb and 500 bp fragments in the 1 Kb ladder are noted with arrows. Lanes 2 through 5 are KAPA products from libraries made from increasing amounts (250 to 400 ng) of fragmented human male DNA (average size of fragment was 500 bp).



VI. Technical Support

For help with any of our products, please contact NuGEN Technical Support at 650.590.3674 (direct) or 888.654.6544, option 2 (toll-free, US only). You may also send faxes to 888.296.6544 (toll-free) or email techserv@nugen.com.

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216 (Fax) or email europe@nugen.com.

In all other locations, contact your NuGEN distributor for technical support.

VII. Appendix

A. Preparation of Encore SP Rapid Library Systems Libraries for Cluster Generation on Illumina Systems

Guidance for preparation of Encore SP Rapid Library Systems libraries for cluster generation is provided below based upon the final library concentration. Using reduced volume, modified recipes may enable you to save an aliquot of the library for later studies.

Follow the standard protocol for creation of library denaturation mix if you would like to use the entire aliquot of Encore SP Rapid Library Systems library in the sequencing reaction.

We recommend following the half-volume protocol for the library denaturation mix if you would prefer to retain half the Encore SP Rapid Library Systems library aliquot for later use.

If your final library concentration is between 1.11 and 1.95 nM, we recommend following the instructions below under the header 'For Library Concentrations Lower than 2 nM.'

For Library Concentrations of 2 nM or Greater

1. Dilute libraries to a concentration 2 nM using Library Dilution Buffer Mix (LD1).
2. Mix the NaOH and the 2 nM Encore SP Rapid Library Systems library together in a microcentrifuge tube according to the volumes shown in Table 8.

Table 8. Library Denaturation Mix for 2 nM libraries

REAGENT	ILLUMINA STANDARD PROTOCOL	HALF-VOLUME PROTOCOL
0.1N NaOH	10 µL	5 µL
2 nM Encore SP Rapid Library Systems Library	10 µL	5 µL
Initial volume of Library Denaturation Mix for 2 nM libraries	**20 µL**	**10 µL**

3. Incubate the tube at room temperature for 5 minutes.
4. Add pre-chilled HT1 reagent (from the Illumina Sequencing Reagent kit) to the microcentrifuge tube as indicated in Table 9.

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Table 9. Final Library Denaturation Mix for 2 nM libraries

REAGENT	ILLUMINA STANDARD PROTOCOL	HALF-VOLUME PROTOCOL
Volume of Library Denaturation Mix already in tube	20 µL	10 µL
Pre-chilled HT1	980 µL	490 µL
Final volume of library denaturation mix	**1000 µL**	**500 µL**

The final concentrations of NaOH and library in the library denaturation mix are the same regardless of which volume protocol is used, as indicated in Table 10.

Table 10. Final NaOH and DNA concentrations in the Library Denaturation Mix for 2 nM libraries

REAGENT	ILLUMINA STANDARD PROTOCOL	HALF-VOLUME PROTOCOL
NaOH	1.0 mM	1.0 mM
Encore SP Rapid Library Systems library	20 pM	20 pM

For Library Concentrations Lower than 2 nM

If the final concentration of the Encore SP Rapid Library Systems library is lower than 2 nM (between 1.11 and 1.95 nM), we recommend using a higher concentration of NaOH (0.5N instead of 0.1N) in the library denaturation mix and adding distilled water to make up the difference in volume.

5. Calculate the volume of library required using the following formula:

   Library volume (µL) = 10 ÷ library concentration (nM)

   For example, if the final Encore SP Rapid Library System library concentration is 1.5 nM, the required volume is 10 ÷ 1.5 nM = 6.67 µL.

6. Aliquot the calculated volume of Encore SP Rapid Library System library into a microcentrifuge tube and add 0.5N NaOH and distilled water as required to generate 10 µL of Library Denaturation Mix, as shown in Table 11.

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Table 11. Library Denaturation Mix for <2 nM libraries

REAGENT	<2 nM LIBRARY PROTOCOL
0.5N NaOH	1.0 µL
1.11 to 1.95 nM Encore SP Rapid Library Systems library	Volume (µL) = 10 ÷ library concentration (nM)
dH_2O	To 10 µL final volume
Initial volume of Library Denaturation Mix for <2 nM libraries	**10 µL**

7. Incubate the tube at room temperature for 5 minutes.
8. Add pre-chilled HT1 reagent (from the Illumina Sequencing Reagent kit) to the microcentrifuge tube as indicated in Table 12.

Table 12. Final Library Denaturation Mix for <2 nM libraries

REAGENT	<2 nM LIBRARY PROTOCOL
Volume of Library Denaturation Mix already in tube	10 µL
Pre-chilled HT1	490 µL
Final volume of library denaturation mix for <2 nM libraries	**500 µL**

The final concentrations of NaOH and library in the library denaturation mix are as indicated in Table 13.

Table 13. Final NaOH and library concentrations in the Library Denaturation Mix for <2 nM libraries

REAGENT	<2 nM LIBRARY PROTOCOL
NaOH	1.0 mM
Encore SP Rapid Library Systems library	20 pM

VII. Appendix

B. Barcode Sequences

Table 14. Barcode sequences for dedicated read (DR) adaptors

LIGATION ADAPTOR MIX	BARCODE SEQUENCE
L2V9DR-BC1	AAGGGA
L2V9DR-BC2	CCTTCA
L2V9DR-BC3	GGACCC
L2V9DR-BC4	TTCAGC
L2V9DR-BC5	AAGACG
L2V9DR-BC6	CCTCGG
L2V9DR-BC7	GGATGT
L2V9DR-BC8	TTCGCT
L2V9DR-BC9	ACACGA
L2V9DR-BC10	CACACA
L2V9DR-BC11	GTGTTA
L2V9DR-BC12	TGTGAA
L2V9DR-BC13	ACAAAC
L2V9DR-BC14	CACCTC
L2V9DR-BC15	GTGGCC
L2V9DR-BC16	TGTTGC

VII. Appendix

C. Frequently Asked Questions (FAQs)

Q1. What kind of sequencing primers can I use with your library?
The Encore SP Rapid Library Systems are designed for use with the standard Illumina sequencing primers for both single end and paired-end sequencing applications.

Q2. Can the Encore SP Rapid Library Systems be used with paired-end sequencing?
Yes, they can be used for both single end and paired-end sequencing. Special consideration should be given to the expected insert size in the paired-end assay.

Q3. How much material should I load for cluster generation?
Please follow manufacturer's recommendations and see Appendix A 'Preparation of Encore SP Rapid Library Systems Libraries for Cluster Generation on the Illumina System.'

Q4. Do the Encore SP Rapid Library Systems work with the Illumina Cluster Station (predecessor of the cBot instrument)?
Yes, the Encore SP Rapid Library Systems are also compatible with the Illumina Cluster Station.

Q5. I don't have access to a Covaris instrument. Can I use alternative fragmentation methods?
We have evaluated only Covaris-fragmented DNA during the development of the Encore SP Rapid Library Systems. Other mechanical means of fragmentation, such as nebulization, may be suitable as well.

Q6. How does your protocol improve the efficiency of ligation and avoid adaptor dimer formation?
The Encore SP Rapid Library Systems use optimized chemistries to increase the efficiency of blunt-end adaptor ligation and minimize the amount of adaptor dimer in the library.

Q7. Does NuGEN provide reagents for performing the fragmentation step of the protocol?
We suggest using the Covaris instrument, as indicated in the "materials" section of this user guide. NuGEN does not provide the reagents used in the fragmentation steps.

VII. Appendix

Q8. Are the libraries from the Encore SP Rapid Library Systems compatible with downstream target capture methods like Agilent's SureSelect or Nimblegen's SeqCap EZ?
No, the libraries from the Encore SP Rapid Library Systems do not yield enough library material to be compatible with downstream target capture methods such as Agilent's SureSelect and Nimblegen's SeqCap EZ kits. These protocols usually require a minimum of 500 ng library input. Customers who wish to perform target selection on their samples should use the Ovation SP Ultralow Library Systems (Part Nos. 8033 and 8034) together with the Encore Target Capture Module (Part No. 0332) to prepare samples for target selection with the Agilent and Nimblegen products.

Q9. How can gel purification be eliminated from the workflow and still prevent adaptor dimer formation?
The Encore SP Rapid Library Systems workflow uses bead-based purification and efficient primer design, thus eliminating the need for gel-based purification.

Q10. Can I use an Agilent 2100 Bioanalyzer or a Thermo Scientific NanoDrop Spectrophotometer to determine the final concentration of an Encore SP Rapid Library Systems library and use this measurement to calculate how much library I need for cluster generation?
No. We do not recommend using the Agilent 2100 Bioanalyzer or the Thermo Scientific NanoDrop Spectrophotometer to quantify the Encore SP Rapid Library Systems libraries. You must quantitate these libraries using qPCR. We recommend the KAPA Biosystem Library Quantification kits. Failure to quantitate the Encore SP Rapid Library Systems libraries by qPCR will result in inaccurate concentration readings, which in turn will lead to low cluster density and poor quality sequencing results.

Q11. Can the Encore SP Rapid Library Systems kits be used on the Mondrian SP+ Workstation?
Yes. The Encore SP Rapid Library Systems kits can be used with both the Mondrian SP and SP+ Workstation. When using the Mondrian SP+ Workstation, select the correct Encore SP Rapid protocol.

VII. Appendix

D. Update History

This document, the Encore SP Rapid Library Systems user guide (M01295 v5) is an update to address the following topics.

Description	Section	Page(s)
Removed references to integration with Ovation RNA-Seq System V2 and Ovation RNA-Seq FFPE System (Part Nos. 7102 and 7150)	I.A., VII.C.	3, 29
Updated Table 3	IV.E.	15
Updated Mondrian SP Cartridge Loading Instructions	IV.E.	18–19
Changed loading volume for D ports	IV.E	19
Updated Library Collection from Mondrian SP Cartridge section	IV.E.	20–21

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